# Molecular motors: **Single-molecule mechanics**

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**Novel techniques are revealing the movements and forces associated with single interactions of motor proteins, such as myosin and kinesin, and also of processive enzymes, such as RNA polymerase.**

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Current Biology 1996, Vol 6 No 4:392–394

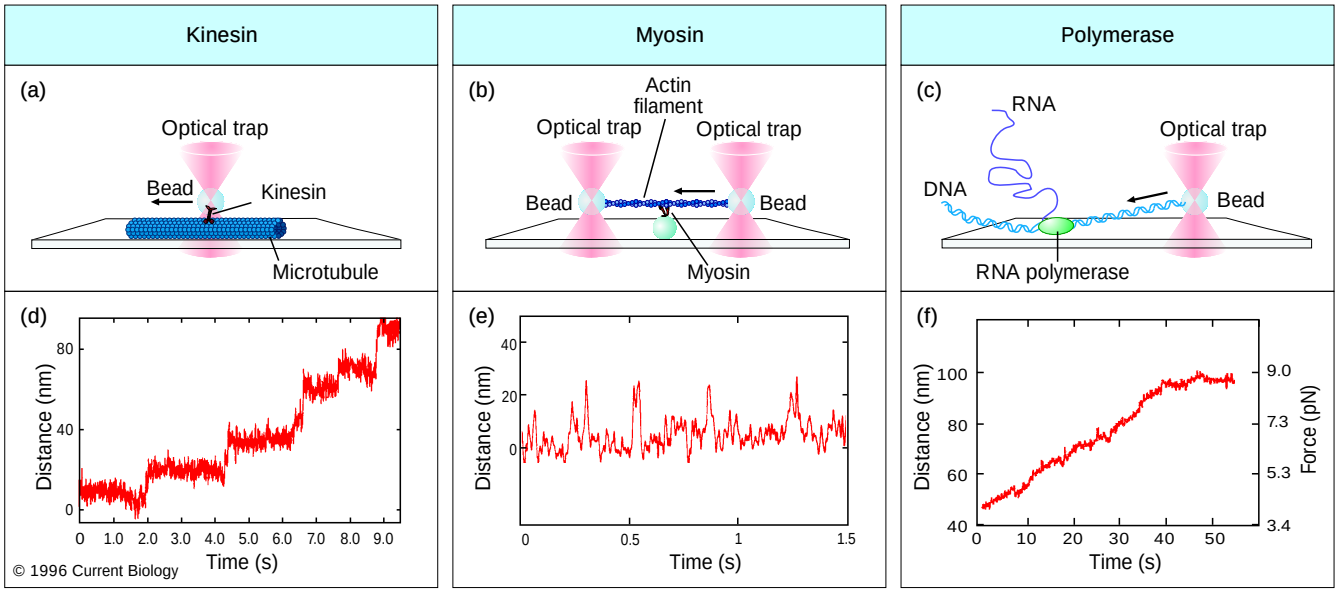


Myosin, kinesin and dynein are the best known examples of molecular motors, distinguished in part by the molecular track along which they move — either actin, in the case of myosin, or microtubules, in the case of kinesin and dynein. Molecular motors do a variety of jobs: moving vesicles, causing muscles to contract, separating chromosomes, and so on. But movement is also an integral part of the function of other proteins, such as DNA-binding proteins that move along DNA, replicating or transcribing as they go, and of course the ribosome, which moves mRNA along during protein synthesis. In the last two years, the combination of *in vitro* motility assays and new micro-mechanical techniques, many of them developed in the laboratories of Steven Block, James Spudich and Toshio Yanagida, has allowed single-molecule mechanical measurements to be made on kinesin, myosin and an RNA polymerase.

Kinesin and myosin are powered by ATP hydrolysis in a cyclic interaction with a microtubule or actin filament, but they differ in the fraction of the cycle they spend attached to their polymer track, a parameter known as the duty ratio. This difference is related to their respective functions. The duty ratio is high for kinesin, a 'porter' which carries vesicles along microtubules for long distances. It is low for muscle myosin, a 'rower' which, when assembled in a muscle thick filament, must pull only briefly on a thin (actin) filament sliding past, so as to avoid drag [1]. The new single-molecule mechanics has given values for the key mechanical characteristics of single interactions, in particular the 'step size' and the force. The definition of step size used here is the average distance a tethered motor protein would move a free filament or microtubule in a single productive interaction.

Several major questions may now be tackled. Does the step involve a conformational change ('power stroke') in the motor protein? This has been supposed to be the case for myosin for nearly 30 years, and Ivan Rayment and his colleagues have proposed a mechanism based on the myosin crystal structure (reviewed in [2]; recently revised

**Figure 1**




Optical tweezers experiments on: (a,d) kinesin [9]; (b,e) myosin [13]; (c,f) RNA polymerase [16]. In the lower panels the ordinate is the distance moved by a trapped bead.

[3]). Or is the step just a monomer-to-monomer transition along the filament or microtubule, resulting from biased thermal motion ('thermal ratchet') [4]? Is the chemomechanical coupling tight, each ATP hydrolysed resulting in a step (1:1 coupling)? Or is it loose, entailing under some conditions the hydrolysis of several ATPs per productive step (>1:1 coupling), or can there even be several steps for each ATP hydrolysed (1:many coupling) [5,6]?

The 'optical tweezers' technique of Arthur Ashkin (reviewed in [7,8]) has been at the heart of most of the recent measurements. In this technique, a silica or polystyrene bead, 0.5–1.0 μm in diameter, is trapped at the focus of a laser beam. Small movements of the bead away from the centre of the trap (detected by interferometric or imaging techniques) are accompanied by a roughly linearly increasing force, in the piconewton range, acting in the direction back towards the centre of the trap. For kinesin, the experimental approach has been to attach the motor protein to beads, at a density of one kinesin molecule per bead [9]. Microtubules are stuck to a coverslip and observed using differential interference microscopy. If a kinesin-containing bead is picked up in an optical trap and placed on a microtubule, the bead starts to move down the microtubule (Fig. 1a).

When the movement of the bead is recorded at high (physiological) ATP concentration and at low load (weak trap), movement appears to be continuous, but autocorrelation analysis shows a peak at 8 nm, the tubulin dimer separation along a protofilament. Under these conditions, steps are masked by the thermal (Brownian) motion of the bead, but when the ATP concentration is lowered so that movement is temporarily arrested, steps can be discerned at 8 nm intervals. The steps become still more apparent if the trap is made stronger, thus reducing the thermal noise, and also reducing velocity (Fig. 1d). The force at the point where movement is just stalled was found to be 5–6 pN. In a second study, somewhat higher forces, 5–10 pN, were recorded in isometric experiments in which movement was inhibited by a feedback system [10]. In these latter experiments the rise in force showed a fine structure, which may arise from the underlying mechanism of the step: kinesin contains two heads (both of which may be required for movement), and is thought to move along a protofilament 'hand over hand'.

Qualitative evidence about coupling can be derived from the observation that, at low ATP concentrations, a step is not preceded by a burst of interactions, which would be predicted by a 1:many scheme. At high ATP concentrations, information about coupling can be derived from fluctuation analysis [11]. The variation in position with time rises as the square root of the number of steps, but also depends on the complexity of the underlying kinetic scheme and on the degree of coupling. The experimental results suggest that models in which coupling is very loose (>>1 ATP per step) can also be discarded. Another approach is to study the force–velocity relation at limiting or saturating ATP concentrations [12]. The two curves differ in maximum velocity, but they are both linear. A theoretical argument shows that, in this case, the effect of increased load must be to reduce the coupling efficiency, either by reducing the size of the step or, more likely, by introducing a number of unproductive ATP cycles, and there is some evidence that, near the stalling point, kinesin fluctuates between one site and the next.

Making well-controlled measurements of single interactions in the myosin system is more taxing than for kinesin, for three reasons. First, myosin spends a sizeable fraction of its cycle detached from actin, so the occurrence of an interaction is unpredictable. Second, filamentous (F) actin is a good deal more flexible than a microtubule, and extraneous compliance can distort the results. And third, it is known that the interaction between actin and myosin is sensitive to their relative orientation. The problem of actin filament compliance was tackled by stretching a filament taut between two beads (each held by optical tweezers), bringing the centre of the filament into contact with a projection coated with myosin, and recording the movement of one of the beads (Fig. 1b) [13].

At a density of myosin that supports continuous motion, stepwise movements of about 11 nm can sometimes be seen, with a complex onset. At still lower densities, records show individual interactions (Fig. 1e). Unlike kinesin, these are short-lasting events, terminated by the detachment of myosin after ATP binding, when the bead is pulled back to the centre of the trap. The average step size was again 11 nm. Under isometric conditions (using a feedback system), the force exerted was 3–4 pN. As with kinesin, these steps were most easily seen at low ATP concentrations. The duration, but not the size, of the events was ATP-dependent, suggesting that transition rates between attached states are load-dependent. However, uncertainty about orientation effects means that these figures should be taken as lower limits. Also as with kinesin, there was no obvious clustering of interactions before a step at low ATP concentrations, again suggesting that the 1:many model is incorrect. The energetics of the interactions suggested the coupling was close to 1:1 at maximum efficiency.

The orientation problem was overcome in another study by using a microneedle assay, in which an actin filament attached to one end of the needle interacted with myosin filaments. Synthetic bipolar myosin filaments were used, in which the surface head density had been reduced by combining in a co-polymer intact myosin and a 'rod' myosin fragment, from which the motor domain had been removed [14]. An advantage of this system is that the end

of the actin filament is kept bound, and the thermal noise presumably damped, by the myosin molecules in the wrong orientation at the distal end of the myosin filament. The relation between force and distance was determined, and shown to be non-linear, with a low slope at high force. The average step size was 17 nm and the maximum force was 6 pN. However, a clustering of events was detected, and the results were taken to support a 1:many model [14].

These first two studies of single-molecule interactions of the myosin motor, using complementary techniques, provided values of the step size and force which were in reasonable agreement. However, a more recent study [15] using optical tweezers has produced lower values. It was shown that the previous studies had overlooked a large population of very small interactions hidden in the noisy baseline, leading to a bias towards large values. When corrected for this effect, the average value of the step size was 4 nm, and that of the force was 2 pN. Similar results were obtained for both two-headed myosin and the single-headed motor domain (subfragment-1). The step size of 4 nm is close to the power stroke predicted from the structure of myosin [2], but it is also close to the actin spacing of 5.5 nm, favoured by thermal ratchet mechanisms. The low values of step size and force would mean that the work done per step is also low, and thus keep alive the possibility of several steps per ATP hydrolysed (that is, 1:many coupling).

The first DNA-binding protein to be investigated by micro-mechanical techniques is RNA polymerase [16]. RNA polymerase moves two or three orders of magnitude more slowly than kinesin or myosin, with a maximum mRNA production rate of about 15 bases per second, giving a velocity of about 5 nm per second. Another difference is that the polymerase reaction is powered by the free energy of condensation of the nucleoside triphosphates (NTPs), rather than by ATP hydrolysis. In the experiments, a transcribing complex was first formed and stabilized by NTP depletion. The ternary complex — RNA polymerase, DNA and RNA transcript — was then tethered to a coverslip (in most cases it seemed that the polymerase was bound to the surface). Derivatized polystyrene beads were then introduced, and it was found that these stuck to the untranscribed end of the DNA (Fig. 1c). Restarting the reaction with NTPs resulted in the bead being pulled away from the centre of the trap (Fig. 1f). Individual steps corresponding to the base separation of 0.34 nm in the double helix were not detected, presumably because they were swamped by thermal noise. The stall force was higher than for the other molecular motors, about 13 pN.

Is there a common mechanism among motor proteins? It is too early to say. The major issue of a conformational change versus a thermal ratchet mechanism is still unsettled, as is the related issue of coupling. Is the end in sight? Given the crystal structures of myosin and actin determined by Rayment and Holmes and their colleagues (and with kinesin not far behind), detailed biochemical kinetic schemes, site-directed mutagenesis and now single-molecule mechanics, all we need is single-fluorophore monitoring of ATP hydrolysis (soon to come [17]) to settle the coupling issue, and a real-time single-molecule imaging technique to look for conformational changes.

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